HHS Public Access

Author manuscript

Pharmacol Biochem Behav. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Pharmacol Biochem Behav. 2015 August ; 135: 83–89. doi:10.1016/j.pbb.2015.05.016.

Adenosinergic regulation of binge-like ethanol drinking and associated locomotor effects in male C57BL/6J mice

Brandon M Fritz and **Stephen L Boehm II**

Indiana Alcohol Research Center, Department of Psychology, Indiana University – Purdue University Indianapolis, Indiana

Abstract

We recently observed that the addition of caffeine (a nonselective adenosine receptor antagonist) to a 20% ethanol solution significantly altered the intoxication profile of male C57BL/6J (B6) mice induced by voluntary binge-like consumption in the 'Drinking-in-the-Dark' (DID) paradigm. In the current study, the roles of A_1 and A_{2A} adenosine receptor subtypes, specifically, in binge-like ethanol consumption and associated locomotor effects were explored. Adult male B6 mice (PND 60-70) were allowed to consume 20% ethanol (v/v) or 2% sucrose (w/v) for 6 days via DID. On day 7, mice received a systemic administration (i.p.) of the A_1 antagonist DPCPX (1, 3, 6 mg/kg), the A_{2A} antagonist MSX-3 (1, 2, 4 mg/kg), or vehicle immediately prior to fluid access in DID. Antagonism of the A_1 receptor via DPCPX was found to dose-dependently decrease binge-like ethanol intake and associated blood ethanol concentrations (p's < 0.05), although no effect was observed on sucrose intake. Antagonism of A_{2A} had no effect on ethanol or sucrose consumption, however, MSX-3 elicited robust locomotor stimulation in mice consuming either solution (p's < 0.05). Together, these findings suggest unique roles for the A_1 and A_{2A} adenosine receptor subtypes in binge-like ethanol intake and its associated locomotor effects.

Keywords

Binge drinking; adenosine; mouse; alcohol; ethanol; Drinking-in-the-Dark

1. Introduction

Binge ethanol (alcohol) consumption, defined by levels of intake resulting in significant intoxication (blood alcohol concentration ≥ 80 mg/dl) within a discrete period of ≤ 2 hrs (NIAAA), is a common form of problematic alcohol use associated with an elevated risk for numerous negative consequences and may even mark progression towards more severe alcohol use problems (Chassin et al., 2002, Viner and Taylor, 2007). Therefore, enhancing

Corresponding Author: Brandon M. Fritz, Department of Psychology, Indiana University – Purdue University Indianapolis, 402 N Blackford St, LD 301, Indianapolis, IN 46202, Phone: 952-994-6683, Fax: 317-274-6756, bmfritz@iupui.edu.

our understanding of the neurobehavioral underpinnings of binge drinking will offer insight on the systems it affects and elucidate the potential for intervention and/or treatment.

Much of the substance abuse literature has given attention to dopamine as a crucial neurotransmitter in drug and alcohol consumption and responses (Di Chiara, 1999, Koob, 1992, Le Foll et al., 2009). However, relatively little acknowledgement has been given to the observation that adenosine signaling has the capacity to regulate the efficiency of dopamine neurotransmission, as well as the release and binding efficiency of other neurotransmitters (Fredholm et al., 2005). This is relevant for the alcohol abuse research field as *in vitro* studies have shown that acute alcohol exposure increases extracellular adenosine levels, indirectly, by inhibition of adenosine's transporter, endonucleoside transporter 1 (ENT1) (Nagy et al., 1990, Allen-Gipson et al., 2009).

Preclinical work with rodents suggests that adenosine signaling, indeed, plays a role in alcohol consumption (Choi et al., 2004, El Yacoubi et al., 2003b, Naassila et al., 2002, Thorsell et al., 2007, Di Bonaventura et al., 2012, Houchi et al., 2013, Arolfo et al., 2004, Adams et al., 2008, Houchi et al., 2008, Nam et al., 2013) and that this relationship may be moderated, in part, by the influence of adenosine mechanisms on alcohol's motor-impairing and sedative effects (Choi et al., 2004, Di Bonaventura et al., 2012, Houchi et al., 2013, Houchi et al., 2008). However, alcohol intakes were largely low-to-moderate in these studies (however, see Houchi et al., 2008) and blood alcohol concentrations following drinking were never assessed. In addition, a significant portion of this work has been limited to knockout models or operant paradigms. Behavioral and quantitative genetic studies in rodents suggest that binge-like alcohol consumption is a distinct phenotype (Fritz et al., 2014b, Bell et al., 2006, Iancu et al., 2013). Therefore, elucidating its neurochemical/biological underpinnings will be necessary to more completely understand this form of excessive alcohol drinking. To our knowledge, no study examining the influence of adenosine receptor pharmacology on voluntary binge-like drinking currently exists. Recently, we demonstrated that the addition of caffeine (a nonselective adenosine receptor antagonist) to a 20% ethanol solution significantly altered the intoxication profile of male C57BL/6J (B6) mice induced by voluntary binge-like consu1mption via the 'Drinking-in-the-Dark' (DID) paradigm (Fritz et al., 2014a). The DID paradigm has been validated as a 'binge' paradigm as mice will consistently drink alcohol to reach blood alcohol concentrations in excess of 80-100 mg/dl in a short period of 2 hours (aligning with the aforementioned NIAAA definition of binge drinking) and demonstrate significant motor and cognitive impairment as a result of alcohol consumption in this paradigm (Sprow and Thiele, 2012, Fritz et al., 2014a). Specifically, we observed that the addition of caffeine attenuated ataxia and sedation induced by voluntary binge alcohol consumption, although caffeine did not influence alcohol-induced anxiolysis, memory interference, or alcohol intake. These findings suggest that nonspecific adenosine receptor antagonism via caffeine influences specific facets of binge-like alcohol intoxication in the DID model, thus implicating a role for adenosine signaling in these behaviors. Because caffeine acts upon the adenosine system as a nonselective antagonist (Fredholm et al., 1999), it is unclear how specific adenosine receptor subtypes might influence binge-like alcohol drinking or resultant intoxication as effects at one receptor may compete with those at another. As such, we chose

to explore the role of the A_1 and A_{2A} receptor subtypes, specifically in binge-like alcohol intake of male B6 mice via the DID model and its locomotor consequences.

2. Methods

2.1 Animals

Adult male (PND 56 ± 3) B6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and allowed 10-14 days to acclimate the colony room and single housing prior to experimentation. Animals were maintained on a 12-hour reverse light/dark cycle with lights OFF at 0700 with temperature and humidity held constant near 20° C and 50%, respectively. Food and water were available *ad libitum*. Principles of laboratory animal care were followed and experiments were performed under a protocol approved by the IUPUI School of Science Institutional Animal Care and Use Committee.

2.2 Drugs and Drinking Solutions

The adenosine A_1 receptor antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) and the A_{2A} receptor antagonist 3,7-Dihydro-8-[(1E)-2-(3-Methoxyphenyl)ethenyl]-7-methyl-3-[3-(phosphonooxy)propyl-1-(2-propynyl)-1H-purine-2,6-dione disodium salt hydrate (MSX-3) were both obtained from Sigma Aldrich (St. Louis, MO). Vehicle for DPCPX was a maximal concentration of 30% (v/v) 0.1 M NaOH in sterile physiological saline for the highest administered dose (6 mg/kg). For MSX-3, the maximal concentration of NaOH was 16% (v/v) for the highest dose administered (4 mg/kg). The vehicle control groups had the respective maximal concentration of NaOH in saline to control for any effects the highest concentration of NaOH may have had on drinking or locomotor activity. DPCPX is 1,000-fold more selective for the A_1 receptor over the A_{2A} receptor (Fredholm and Lindström, 1999). MSX-3 is a prodrug for MSX-2, which has demonstrated 100-fold selectivity for A_{2A} over A_1 in both rat and human tissue, with no activity at A_{2B} or A_3 subtypes (Sauer et al., 2000, Solinas et al., 2005). For drinking solutions, ethanol (190 proof; Pharmco Inc., Brookfield, CT) was diluted to 20% (v/v) in tap water and sucrose (Sigma-Aldrich, St. Louis, MO) was dissolved in tap water to 2% (w/v).

2.3 Drinking in the Dark

We used a DID procedure slightly modified from the original version (Rhodes et al., 2005). Beginning 3 hours into the dark cycle, singly housed male B6 mice had their water bottles replaced by a 10 ml sipper tube containing either an alcohol or sucrose solution for 2 hours. During this period, the only fluid available was the designated test fluid. As is common in preclinical alcohol studies, sucrose consumption was assessed in addition to ethanol to evaluate whether either antagonist influenced the intake of a natural reward/reinforcer, allowing for the determination of whether the drugs' effects were ethanol-specific and simply did not influence hedonic processes (Sprow and Thiele, 2012).

2.4 Home Cage Locomotor Activity Monitors

Details concerning the exact monitors (Columbus Instruments Inc., Columbus, OH) used in the current study were previously published (Linsenbardt and Boehm, 2012). Each day, locomotor activity was measured in each mouse's home cage during the 2 hr DID session.

Monitoring activity in this manner allowed for determination of whether the effects of either compound on locomotion could account for fluid intake differences. Furthermore, locomotor activity during alcohol intoxication is also considered a relevant phenotype to address as it reflects sensitivity to alcohol's motor-impairing or – stimulating effects; phenotypes that have been associated with a propensity for high/low alcohol consumption (Colombo et al., 1998, Risinger et al., 1994, Fritz et al., 2013, Kurtz et al., 1996).

2.5 Procedure

Mice acquired ethanol (n = 79) or sucrose (n = 75) drinking via DID over 6 days. Prior to drug challenge on day 7, mice were counterbalanced for ethanol or sucrose consumption as well as locomotor activity prior to drug dose assignment, and then weighed and injected with DPCPX (0, 1, 3, 6 mg/kg, i.p.) or MSX-3 (0, 1, 2, 4 mg/kg, i.p.) immediately prior to fluid access. This study employed a between-subjects design where each mouse had access to only *either* sucrose or ethanol during DID and received a single injection of the assigned drug on day 7. Fluid intakes were recorded every 30 minutes by removing the tube from the cage and reading the volume at eye level. Additional tubes filled with ethanol and sucrose were placed on empty cages and were also read every 30 minutes to control for the spillage produced by multiple readings during the session. The average leakage volumes for ethanol and sucrose were controlled for in the analysis of fluid intakes. Periorbital blood samples were taken from mice that had access to ethanol immediately following the 2 hr access period for later blood alcohol level determination via an AM1 Analox Alcohol Analzyer (Analox Instruments, Lunenburg, MA). Measuring blood alcohol content is crucial to determine whether mice 1) are indeed ingesting the alcohol solution rather than causing it to spill out into the cage and 2) verify that high levels of intake are indeed producing BECs that are considered to be 'binge' levels (≥ 80 mg/dl) in vehicle-treated mice using the DID paradigm.

2.6 Statistical Analysis

Ethanol and sucrose data were analyzed separately. Intake and locomotor data during acquisition were analyzed via repeated measures ANOVA with dose and day as factors. Intake and locomotion data on day 7 were also analyzed via repeated measures ANOVA, but with dose and time (each 30 min bin) as factors. Neuman-Keuls post-hoc statistics were run where applicable.

3. Results

3.1 Acquisition (days 1-6)

Drinking and locomotor activity data during DID acquisition over days 1-6 for alcohol and sucrose groups are represented in Figure 1. Dose groups were counterbalanced so that they did not differ in alcohol/sucrose intake or locomotor activity during this period (all p's > 0.17).

3.2 Test Day

3.2.1 Alcohol intake and locomotor activity—Systemic administration of the A_1 antagonist DPCPX was found to significantly dose-dependently reduce alcohol intake [$F_{3,34}$

= 5.34, $p < 0.01$] with the 3 and 6 mg/kg doses being effective relative to vehicle (post-hoc p 's < 0.05; Figure 2A). Although the dose × time interaction was not statistically significant ($p > 0.05$), we also analyzed intake during the first 30 min portion of DID drinking separately on the basis of previous observations that male B6 mice consume alcohol most heavily within this early phase of DID (Wilcox et al., 2014, Linsenbardt and Boehm, 2014). The 6 mg/kg dose effectively reduced early alcohol drinking [$F_{3,34} = 87.71$, $p < 0.01$], an effect that appears to be largely responsible for its reduction in overall alcohol intake. Furthermore, DPCPX significantly reduced attained BACs [$F_{3,34} = 3.01$, $p < 0.05$] with the 6 mg/kg dose reducing BACs below vehicle levels (post-hoc $p < 0.05$; Figure 3A). Locomotor activity during alcohol drinking in DID was unaffected by DPCPX ($p > 0.05$; Figure 2C) and dose did not interact with time ($p > 0.05$).

In contrast, the A_{2A} antagonist MSX-3 had no effect on early (first 30 min) or overall alcohol intake (p's > 0.05; Figure 2B) and BACs were also unaffected ($p > 0.05$; Figure 3B). However, MSX-3 dose-dependently increased locomotion during ethanol consumption in DID [$F_{3,37} = 7.96$, $p < 0.001$] with the 2 and 4 mg/kg doses producing significant stimulation (post-hoc p's < 0.05; Figure 2D). The dose × time interaction was not statistically significant ($p > 0.05$).

3.2.2 Sucrose intake and locomotor activity—Antagonism of the A_1 adenosine receptor via systemic administration of DPCPX did not alter early (first 30 min) or overall sucrose intake and dose did not interact with time (p's > 0.05; Figure 4A). However DPCPX did stimulate locomotor activity [$F_{3,31} = 4.07$, $p < 0.05$] with the 1 and 3 mg/kg doses being effective (post-hoc p 's < 0.05; Figure 4B). A significant dose × time interaction [$F_{9,93} = 2.62$, $p < 0.01$] also revealed that these effects occurred early (30 min) and late (120 min) in the DID test (p's < 0.05).

Antagonism of the A_{2A} adenosine receptor via systemic administration of MSX-3 did not alter early (first 30 min) or overall sucrose intake and dose did not interact with time (p's > 0.05; Figure 4B). Overall locomotor activity was also found to be stimulated by MSX-3 during sucrose drinking in DID [$F_{3,34} = 5.02$, $p < 0.01$] with only the 4 mg/kg dose producing significant stimulation ($p < 0.01$). A significant dose × time interaction [$F_{9,102} = 3.73$, $p < 0.001$] also revealed that all doses of MSX-3 stimulated locomotion in the first 30 minutes relative to vehicle-treated mice with the 4 mg/kg dose having the most robust effect (p 's < 0.05). Furthermore, only the 4 mg/kg dose effectively promoted locomotor stimulation at the 60 min time point ($p < 0.01$).

4. Discussion

The present study found that antagonism of the adenosine A_1 receptor significantly reduced alcohol consumption below binge levels (6 mg/kg dose of DPCPX reduced mean BAC below 80 mg/dl), however, A_{2A} antagonism had no effect. These observations were specific to binge-like alcohol consumption as sucrose intake was not affected by either compound. Furthermore, antagonism of the A_{2A} receptor significantly stimulated locomotor activity in alcohol- and sucrose-consuming mice, with a more robust effect in alcohol-consuming mice. However, only mice consuming sucrose demonstrated locomotor stimulation via A_1

antagonism. Together, these results suggest different roles for these receptors in binge-like alcohol drinking and associated locomotor activity.

Previous preclinical studies have demonstrated a role for adenosine signaling in alcohol consumption. Although there are 4 known adenosine receptors, the most studied are the 2 prominent adenosine receptor subtypes in the central nervous system, the A_1 and A_{2A} receptors, and activation of these subtypes has been shown to significantly influence the release and/or binding efficiency of many neurotransmitters (Fredholm et al., 2005). As such, these receptor subtypes have been the primary focus of study in alcohol abuse research in addition to ENT1. Genetic deletion of ENT1 in mice, presumably shunting alcohol-induced increases in extracellular adenosine, elevates alcohol consumption in a 2-bottle choice paradigm (Choi et al., 2004). Pharmacological blockade of A_{2A} receptors has been shown to both increase (Arolfo et al., 2004, Di Bonaventura et al., 2012) and decrease (Adams et al., 2008, Thorsell et al., 2007) alcohol responding/consumption in rats. One recent study in mice suggests that goal-directed behavior in operant responding for alcohol may be mediated in part by the dorsomedial striatum as local A_{2A} antagonism increases responding for alcohol (Nam et al., 2013). Conversely, pharmacological A_{2A} activation via agonists reduces alcohol responding/consumption in operant paradigms as well as 2-bottle choice drinking in rats and mice (Di Bonaventura et al., 2012, Houchi et al., 2013, Houchi et al., 2008). Furthermore, one study with A_{2A} knockout mice generated on a CD1 background found increased alcohol consumption in a 2-bottle choice paradigm (Naassila et al., 2002), however a later study with knockouts generated on a B6 background found no effect of A_{2A} deletion on alcohol intake (Houchi et al., 2008). When a number of these studies evaluated the role of the A_1 receptor, however, genetic deletion or pharmacological antagonism was found to have no effect on responding for/consumption of alcohol (Adams et al., 2008, Arolfo et al., 2004, Houchi et al., 2013).

Collectively, these observations are strikingly different from the findings of the current study. We found no evidence of A_{2A} antagonism on either alcohol or sucrose intake in the DID paradigm and an alcohol-specific decrease in intake via A_1 blockade with the 6 mg/kg dose of DPCPX significantly reducing both alcohol intake and BAC. Although the 3 mg/kg dose of DPCPX was found to decrease total alcohol intake over the 2 hr session, this did not translate to a significantly reduced BAC level relative to vehicle. One reason for this may be that the most prominent and discernable dose effect of DPCPX is clearly within the first 30 minutes of fluid access (Figure 2A). As mentioned earlier, only the 6 mg/kg dose significantly reduced alcohol intake in this early time bin. Therefore, it may not be surprising that the observed effects on BAC mirror the major drug effect, which is clearly demonstrated within the first 30 minute time bin. Although neither compound was found to influence sucrose intake, it is important to note that mice did consume larger volumes of the sucrose solution than the ethanol solution in this study, raising the possibility of whether a ceiling effect existed for sucrose-drinking mice. Our group has previously used a higher concentration of sucrose (5% w/v) in the DID paradigm and found that mice consumed far more fluid (~120 ml/kg) (Melón and Boehm II, 2011) than what was observed with 2% sucrose in the present study (~70 ml/kg). Thus, although we cannot rule out the possibility that the 2% sucrose solution may have been too rewarding to observe an effect of either antagonist, we feel that this is unlikely in light of this previous finding.

To our knowledge, this is the first observation of A_1 receptor manipulation influencing alcohol consumption. There are a number or reasons why our findings do not appear to align with those of previous studies. First, although prior work suggests a prominent role for the A_{2A} receptor in alcohol seeking and consumption, this may be dependent on the rodent genotype. As mentioned above, Houchi et al. (2008) were not able to replicate the elevated alcohol intake of A_{2A} knockout mice bred on a CD1 background when they instead bred mice on a B6 background. This may also offer an explanation as to why A_{2A} blockade was ineffective in manipulating the binge-like alcohol intake of male B6 mice in the current study. Second, although the BACs of animals were not evaluated in any of the aforementioned studies, the alcohol intake levels observed were largely low to moderate (however, see Houchi et al., 2008) and therefore likely below binge levels (< 80 mg/dl). The current study employed the binge-like drinking DID paradigm and our vehicle control groups consumed > 4.2 g/kg in 2 hrs and reached BACs ≥ 87 mg/dl, on average (Figures 1A and 2). Therefore, A_1 receptors may be more influential in regulating higher, binge-like alcohol intake. In addition, the extent of a subject's alcohol consumption history may influence sensitivity to pharmacological manipulation of A_1 or A_{2A} receptors. In contrast to the current study, these previous studies largely employed alcohol drinking paradigms that spanned the course of weeks. More protracted alcohol consumption such as this may have influenced adenosine receptor makeup/number/sensitivity in a way that mitigated the influence of the A_1 receptor. Indeed, one previous study showed that pre-treatment with DPCPX attenuated the development of rapid tolerance to alcohol's ataxic effect in mice (Batista et al., 2005), suggesting that A_1 receptor activation is involved in the adaptive response to alcohol intoxication. Our lab and others have shown that longer DID access (~2-4 weeks opposed to 1 week) produces ataxic tolerance (Linsenbardt et al., 2011), alterations in associated locomotion (Linsenbardt and Boehm, 2014, Linsenbardt et al., 2011, Fritz et al., 2014a), and the rate of alcohol intake (Wilcox et al., 2014, Linsenbardt and Boehm, 2014), perhaps indicating that the duration of alcohol access in this model may also alter sensitivity to one or both of these compounds. In addition, animals in our study were freely consuming alcohol in their home cage versus operant chambers in other studies. This may reflect different roles for the A_1 and A_{2A} receptors in voluntary alcohol consumption versus alcohol reinforcement, although future studies should more directly address this possibility. In the context of this systemic administration study, it is also important to note that A_1 and A_{2A} receptors are present in the periphery, largely regulating cardiovascular and inflammatory processes (Albrecht-Küpper et al., 2012, Deharo et al., 2012, Williams and Cronstein, 2012, Antonioli et al., 2013). Future efforts employing brain-region specific administration will seek to clarify the extent to which the observed effects are mediated by central or perhaps, peripheral effects. Finally, it is worth noting that evaluating A_1 or A_{2A} agonists as well in the current study could clarify these results further, offering more information on how activity through these receptor subtypes may influence the phenotypes examined. However, we chose to only use antagonists due to the aforementioned sedative properties of adenosine signaling and systemic administration of agonists would raise concerns over competing sedative responses. Moreover, as alcohol has been demonstrated to produce motor impairment through these adenosine receptor subtypes (Dar, 2002, El Yacoubi et al., 2003a), evaluating their systemic effects during alcohol consumption appeared problematic. As mentioned above, however, central administration with

microinjection techniques may offer a more appropriate way to evaluate the effects of adenosine agonists.

The role of adenosine signaling in alcohol's motor effects has been more thoroughly characterized. A_1 receptors, in particular, have been implicated in alcohol-induced ataxia with both systemic and central antagonist/agonist pretreatment reducing/accentuating ataxia following an acute injection of alcohol (Barwick and Dar, 1998, Connole et al., 2004). In addition, activation of the A_1 receptor has been shown to be an important factor in developing tolerance to alcohol's ataxic effects as systemic pretreatment with DPCPX attenuates rapid tolerance development (Batista et al., 2005). A_1 receptors are pervasive throughout the brain with higher concentrations in cortex and hippocampus, although they are very prominent in the cerebellum (Goodman and Synder, 1982, Fastbom et al., 1987), where they likely exert the greatest influence on alcohol-induced ataxia (Dar, 1996; 2002). A_{2A} receptors on the other hand appear to be more influential in alcohol-induced sedation, with antagonist pretreatment or genetic deletion in knockout mice reducing alcohol-induced hypnosis in the loss of righting reflex task (El Yacoubi et al., 2003b, Naassila et al., 2002). A_{2A} knockout mice bred on a CD1 background also demonstrate significantly greater acute locomotor stimulation following a 1.5 g/kg dose of alcohol (i.p.), however, this was not observed in mice bred on a B6 background (Houchi et al., 2008). We observed that both A_1 and A_{2A} antagonism significantly stimulated locomotor activity in mice consuming sucrose, however only A_{2A} antagonism produced significant locomotor stimulation in mice consuming alcohol. The observation of global locomotor stimulation is not surprising as MSX-3 has previously been demonstrated to stimulate locomotion in rats (Müller et al., 1998, Nagel et al., 2003). A_{2A} receptors are highly localized to the striatum (Rosin et al., 1998), a region where dopaminergic input is strongly involved in motor activity (Wickens, 1990). Interestingly, the stimulant effect of MSX-3 appeared to be more efficient in mice consuming alcohol as both the 2 and 4 mg/kg doses produced robust stimulation whereas only the 4 mg/kg dose did so in sucrose-consuming mice. Although the baseline activity of alcohol-consuming mice may have been lower than that of sucrose mice, the increase in activity relative to control mice was nevertheless more robust and the 2 mg/kg dose effectively elicited stimulation. One reason this may be is that binge-like alcohol consumption could have produced an increase in striatal dopamine levels that when coupled with A_{2A} antagonism, resulted in a more pronounced stimulant response. Indeed, voluntary binge-like alcohol consumption has been demonstrated to produce an increase in dopamine levels in the nucleus accumbens (part of the ventral striatum) of male B6 mice (Szumlinski et al., 2007). It is not clear why the low and moderate doses of DPCPX produced locomotor stimulation only in mice consuming sucrose. One possibility is that alcohol consumption may have positively modulated adenosine signaling in some capacity thus, competing with the ability of DPCPX to induce locomotor stimulation. As mentioned above, the A_1 receptor is highly involved in the motor effects of alcohol intoxication and alcohol consumption may therefore have competed with the locomotor-stimulating effect of these doses of DPCPX.

Adenosine signaling interacts with many other neurotransmitter systems (Fredholm et al., 2005), although we speculate that its associative role with dopamine signaling may account for some of effects observed here and elsewhere on alcohol consumption and locomotor activity. The A_1 and A_{2A} receptor subtypes are the most prominent in the central nervous

system and activation of these receptors has been shown to have antagonistic effects on dopamine release and binding potential for D_1 and D_2 receptors (Fredholm et al., 2005, Fuxe et al., 2010). This is in large part because adenosine and dopamine receptors can form functional heterodimers, with A_1 associating with the D_1 receptor and A_{2A} associating with D_2 receptor. Interestingly, one previous study observed that pharmacological activation via the D_1 partial agonist SKF 38393 decreases 1-hr limited access alcohol consumption in male Wistar rats whereas the D_2 agonist quinpirole had no effect (Linseman, 1990). These observations may reflect what was observed in the current study where antagonism of the A_1 receptor may have released adenosinergic inhibition of dopamine binding to the D_1 receptor and thus, attenuated alcohol consumption while no effect of A_{2A} antagonism on binge like alcohol consumption may fall in line with a lack of D_2 receptor influence. However, it should be noted that other studies have shown that pharmacological activation of both D_1 and D_2 receptors can influence alcohol consumption/seeking in rodents (Dyr et al., 1993, Ng and George, 1994, Ingman et al., 2006, Hodge et al., 1997, Silvestre et al., 1996, Bono et al., 1996). Although, these studies employed longer alcohol access periods (4+ hrs) or operant paradigms and may therefore reflect a form of alcohol consumption different from a voluntary binge model.

As A_1-D_1 and A_{2A}-D_2 heterodimers are both found on GABA neurons and glutamate synapses in striatopallidal circuitry (Azdad et al., 2009, Ferré et al., 2002, Franco et al., 2007), this complex dopamine-adenosine interaction has the capacity to regulate basal ganglia output, an effect which may underlie the observed locomotor stimulation in the current study by both antagonists. Furthermore, a previous study found that A_{2A} antagonism specifically in the dorsomedial, but not dorsolateral striatum, increased goal-directed alcohol seeking in mice (Nam et al., 2013), suggesting that this behavior can be regulated in some capacity by striatal adenosine signaling. Although we observed no effect of A_{2A} antagonism in the current study, global antagonism by systemic administration may have produced competing effects in other brain regions. Furthermore, it is also possible that A_1 receptors specifically located in striatal subregions may regulate binge-like drinking, mirroring our findings with systemically administered drug. Future studies aimed at microinjecting antagonists into striatal subregions will explore these possibilities.

In conclusion, we have demonstrated that antagonism of the A_1 receptor produces an alcohol-specific decrease in binge-like intake via the DID model. Although A_{2A} receptor antagonism did not influence alcohol or sucrose intake, it produced locomotor stimulation that was particularly robust in alcohol-consuming mice. These findings suggest that A_1 receptors play a regulatory role in binge-like alcohol consumption whereas A_{2A} receptors influence its locomotor effects during intoxication.

Acknowledgements

This work was funded, in part, by NIAAA Alcohol Training Grant T32 AA07462.

REFERENCES

Adams CL, Cowen MS, Short JL, Lawrence AJ. Combined antagonism of glutamate mGlu5 and adenosine A2A receptors interact to regulate alcohol-seeking in rats. The International Journal of Neuropsychopharmacology. 2008; 11:229–241. [PubMed: 17517168]

Albrecht-Küpper BE, Leineweber K, Nell PG. Partial adenosine A1 receptor agonists for cardiovascular therapies. Purinergic signalling. 2012; 8:91–99. [PubMed: 22081230]

Allen-Gipson DS, Jarrell JC, Bailey KL, Robinson JE, Kharbanda KK, Sisson JH, Wyatt TA. Ethanol blocks adenosine uptake via inhibiting the nucleoside transport system in bronchial epithelial cells. Alcoholism: Clinical and Experimental Research. 2009; 33:791–798.

Antonioli L, Blandizzi C, Pacher P, Haskó G. Immunity, inflammation and cancer: a leading role for adenosine. Nature Reviews Cancer. 2013; 13:842–857.

Arolfo MP, Yao L, Gordon AS, Diamond I, Janak PH. Ethanol Operant Self-Administration in Rats Is Regulated by Adenosine A2 Receptors. Alcoholism: Clinical and Experimental Research. 2004; 28:1308–1316.

Azdad K, Gall D, Woods AS, Ledent C, Ferré S, Schiffmann SN. Dopamine D2 and adenosine A2A receptors regulate NMDA-mediated excitation in accumbens neurons through A2A-D2 receptor heteromerization. Neuropsychopharmacology. 2009; 34:972–986. [PubMed: 18800071]

Barwick VS, Dar MS. Adenosenergic modulation of ethanol-induced motor incoordination in the rat motor cortex. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 1998; 22:587–607. [PubMed: 9682275]

Batista LC, Prediger RD, Morato GS, Takahashi RN. Blockade of adenosine and dopamine receptors inhibits the development of rapid tolerance to ethanol in mice. Psychopharmacology. 2005; 181:714–721. [PubMed: 15983797]

Bell RL, Kimpel MW, Rodd ZA, Strother WN, Bai F, Peper CL, Mayfield RD, Lumeng L, Crabb DW, Mcbride WJ, Witzmann FA. Protein expression changes in the nucleus accumbens and amygdala of inbred alcohol-preferring rats given either continuous or scheduled access to ethanol. Alcohol. 2006; 40:3–17. [PubMed: 17157716]

Bono G, Balducci C, Richelmi P, Koob GF, Pulvirenti L. Dopamine partial receptor agonists reduce ethanol intake in the rat. European Journal of Pharmacology. 1996; 296:233–238. [PubMed: 8904074]

Chassin L, Pitts SC, Prost J. Binge drinking trajectories from adolescence to emerging adulthood in a high-risk sample: predictors and substance abuse outcomes. Journal of consulting and clinical psychology. 2002; 70:67. [PubMed: 11860058]

Choi D-S, Cascini M-G, Mailliard W, Young H, Paredes P, Mcmahon T, Diamond I, Bonci A, Messing RO. The type 1 equilibrative nucleoside transporter regulates ethanol intoxication and preference. Nature neuroscience. 2004; 7:855–861.

Colombo G, Agabio R, Lobina C, Reali R, Vacca G, Gessa GL. Stimulation of locomotor activity by voluntarily consumed ethanol in Sardinian alcohol-preferring rats. European Journal of Pharmacology. 1998; 357:109–113. [PubMed: 9797025]

Connole L, Harkin A, Maginn M. Adenosine A1 Receptor Blockade Mimics Caffeine's Attenuation of Ethanol-Induced Motor Incoordination. Basic & clinical pharmacology & toxicology. 2004; 95:299–304. [PubMed: 15569276]

Dar MS. Mouse cerebellar GABA B participation in the expression of acute ethanol-induced ataxia and in its modulation by the cerebellar adenosinergic A 1 system. Brain research bulletin. 1996; 41:53–59. [PubMed: 8883916]

Dar MS. Mouse Cerebellar Adenosine-Glutamate Interactions and Modulation of Ethanol-Induced Motor Incoordination. Alcoholism: Clinical and Experimental Research. 2002; 26:1395–1403.

Deharo J-C, Mechulan A, Giorgi R, Franceschi F, Prevot S, Peyrouse E, Condo J, By Y, Ruf J, Brignole M. Adenosine plasma level and A2A adenosine receptor expression: correlation with laboratory tests in patients with neurally mediated syncope. Heart. 2012; 98:855–859. [PubMed: 22581734]

Di Bonaventura MVM, Cifani C, Lambertucci C, Volpini R, Cristalli G, Froldi R, Massi M. Effects of A2A adenosine receptor blockade or stimulation on alcohol intake in alcohol-preferring rats. Psychopharmacology. 2012; 219:945–957. [PubMed: 21833502]

Di Chiara G. Drug addiction as dopamine-dependent associative learning disorder. European journal of pharmacology. 1999; 375:13–30. [PubMed: 10443561]

Dyr W, Mcbride WJ, Lumeng L, Li TK, Murphy JM. Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. Alcohol. 1993; 10:207–212. [PubMed: 8507389]

El Yacoubi M, Ledent C, Parmentier M, Costentin J, Vaugeois J-M. Caffeine reduces hypnotic effects of alcohol through adenosine A 2A receptor blockade. Neuropharmacology. 2003a; 45:977–985. [PubMed: 14573390]

El Yacoubi M, Ledent C, Parmentier M, Costentin J, Vaugeois JM. Caffeine reduces hypnotic effects of alcohol through adenosine A2A receptor blockade. Neuropharmacology. 2003b; 45:977–985. [PubMed: 14573390]

Fastbom J, Pazos A, Palacios J. The distribution of adenosine A1 receptors and 5′-nucleotidase in the brain of some commonly used experimental animals. Neuroscience. 1987; 22:813–826. [PubMed: 2825070]

Ferré S, Karcz-Kubicha M, Hope BT, Popoli P, Burgueño J, Gutiérrez MA, Casadó V, Fuxe K, Goldberg SR, Lluis C. Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proceedings of the National Academy of Sciences. 2002; 99:11940–11945.

Franco R, Lluis C, Canela E, Mallol J, Agnati L, Casado V, Ciruela F, Ferre S, Fuxe K. Receptor-receptor interactions involving adenosine A1 or dopamine D1 receptors and accessory proteins. Journal of neural transmission. 2007; 114:93–104. [PubMed: 17024327]

Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacological reviews. 1999; 51:83–133. [PubMed: 10049999]

Fredholm BB, Chen J-F, Masino SA, Vaugeois J-M. Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. Annu. Rev. Pharmacol. Toxicol. 2005; 45:385–412. [PubMed: 15822182]

Fredholm BB, Lindström K. Autoradiographic comparison of the potency of several structurally unrelated adenosine receptor antagonists at adenosine A1 and A2A receptors. European Journal of Pharmacology. 1999; 380:197–202. [PubMed: 10513579]

Fritz BM, Companion M, Boehm SL. “Wired,” Yet Intoxicated: Modeling Binge Caffeine and Alcohol Co-Consumption in the Mouse. Alcoholism: Clinical and Experimental Research. 2014a; 38:2269–2278.

Fritz BM, Cordero KA, Barkley-Levenson AM, Metten P, Crabbe JC, Boehm SL. Genetic Relationship Between Predisposition for Binge Alcohol Consumption and Blunted Sensitivity to Adverse Effects of Alcohol in Mice. Alcoholism: Clinical and Experimental Research. 2014b; 38:1284–1292.

Fritz BM, Grahame NJ, Boehm SL. Selection for high alcohol preference drinking in mice results in heightened sensitivity and rapid development of acute functional tolerance to alcohol’s ataxic effects. Genes, Brain and Behavior. 2013; 12:78–86.

Fuxe K, Marcellino D, Borroto-Escuela D, Guescini M, Fernández-Dueñas V, Tanganelli S, Rivera A, Ciruela F, Agnati L. Adenosine-dopamine interactions in the pathophysiology and treatment of CNS disorders. CNS neuroscience & therapeutics. 2010; 16:e18–e42. [PubMed: 20345970]

Goodman RR, Synder S. Autoradiographic localization of adenosine receptors in rat brain using [3H] cyclohexyladenosine. The Journal of Neuroscience. 1982; 2:1230–1241. [PubMed: 6288896]

Hodge CW, Samson HH, Chappelle AM. Alcohol Self-Administration: Further Examination of the Role of Dopamine Receptors in the Nucleus Accumbens. Alcoholism: Clinical and Experimental Research. 1997; 21:1083–1091.

Houchi H, Persyn W, Legastelois R, Naassila M. The adenosine A2A receptor agonist CGS 21680 decreases ethanol self-administration in both non-dependent and dependent animals. Addiction biology. 2013; 18:812–825. [PubMed: 23301633]

Houchi H, Warnault V, Barbier E, Dubois C, Pierrefiche O, Ledent C, Daoust M, Naassila M. Involvement of A2A receptors in anxiolytic, locomotor and motivational properties of ethanol in mice. Genes, Brain and Behavior. 2008; 7:887–898.

Iancu OD, Oberbeck D, Darakjian P, Metten P, Mcweeney S, Crabbe JC, Hitzemann R. Selection for Drinking in the Dark Alters Brain Gene Coexpression Networks. Alcoholism: Clinical and Experimental Research. 2013; 37:1295–1303.

Ingman K, Kupila J, Hyytiä P, Korpi ER. Effects of aripiprazole on alcohol intake in an animal model of high-alcohol drinking. Alcohol and Alcoholism. 2006; 41:391–398. [PubMed: 16684847]

Koob, GF. Seminars in Neuroscience. Elsevier; 1992. Year. Dopamine, addiction and reward; p. 139-148.

Kurtz DL, Stewart RB, Zweifel M, Li TK, Froehlich JC. Genetic differences in tolerance and sensitization to the sedative/hypnotic effects of alcohol. Pharmacology Biochemistry and Behavior. 1996; 53:585–591.

Le Foll B, Gallo A, Le Strat Y, Lu L, Gorwood P. Genetics of dopamine receptors and drug addiction: a comprehensive review. Behavioural pharmacology. 2009; 20:1–17. [PubMed: 19179847]

Linseman MA. Effects of dopaminergic agents on alcohol consumption by rats in a limited access paradigm. Psychopharmacology. 1990; 100:195–200. [PubMed: 1968278]

Linsenbardt DN, Boehm SL. Role of Novelty and Ethanol History in Locomotor Stimulation Induced by Binge-Like Ethanol Intake. Alcoholism: Clinical and Experimental Research. 2012; 36:887–894.

Linsenbardt DN, Boehm SL. Alterations in the rate of binge ethanol consumption: implications for preclinical studies in mice. Addiction biology. 2014; 19:812–825. [PubMed: 23742054]

Linsenbardt DN, Moore EM, Griffin KD, Gigante ED, Boehm SL 2nd. Tolerance to Ethanol's Ataxic Effects and Alterations in Ethanol-Induced Locomotion Following Repeated Binge-Like Ethanol Intake Using the DID Model. Alcoholism: Clinical and Experimental Research. 2011; 35:1246–1255.

Melón LC, Boehm SL Ii. GABAA receptors in the posterior, but not anterior, ventral tegmental area mediate Ro15-4513-induced attenuation of binge-like ethanol consumption in C57BL/6J female mice. Behavioural brain research. 2011; 220:230–237. [PubMed: 21320533]

Müller CE, Sauer R, Maurinsh Y, Huertas R, Fülle F, Klotz KN, Nagel J, Hauber W. A2A-selective adenosine receptor antagonists: Development of water-soluble prodrugs and a new tritiated radioligand. Drug development research. 1998; 45:190–197.

Naassila M, Ledent C, Daoust M. Low ethanol sensitivity and increased ethanol consumption in mice lacking adenosine A2A receptors. The Journal of neuroscience. 2002; 22:10487–10493. [PubMed: 12451148]

Nagel J, Schladebach H, Koch M, Schwienbacher I, Müller CE, Hauber W. Effects of an adenosine A2A receptor blockade in the nucleus accumbens on locomotion, feeding, and prepulse inhibition in rats. Synapse. 2003; 49:279–286. [PubMed: 12827647]

Nagy LE, Diamond I, Casso D, Franklin C, Gordon A. Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. Journal of Biological Chemistry. 1990; 265:1946–1951. [PubMed: 2298733]

Nam HW, Hinton DJ, Kang NY, Kim T, Lee MR, Oliveros A, Adams C, Ruby CL, Choi D-S. Adenosine Transporter ENT1 Regulates the Acquisition of Goal-Directed Behavior and Ethanol Drinking through A2A Receptor in the Dorsomedial Striatum. The Journal of Neuroscience. 2013; 33:4329–4338. [PubMed: 23467349]

Ng GYK, George SR. Dopamine receptor agonist reduces ethanol self-administration in the ethanol-preferring C57BL/6J inbred mouse. European Journal of Pharmacology: Molecular Pharmacology. 1994; 269:365–374.

Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC. Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiology & behavior. 2005; 84:53–63. [PubMed: 15642607]

Risinger F, Malott D, Prather L, Niehus D, Cunningham C. Motivational properties of ethanol in mice selectively bred for ethanol-induced locomotor differences. Psychopharmacology. 1994; 116:207–216. [PubMed: 7862950]

Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J. Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. The Journal of Comparative Neurology. 1998; 401:163–186. [PubMed: 9822147]

Sauer R, Maurinsh J, Reith U, Fülle F, Klotz K-N, Müller CE. Water-soluble phosphate prodrugs of 1-propargyl-8-styrylxanthine derivatives, A2A-selective adenosine receptor antagonists. Journal of medicinal chemistry. 2000; 43:440–448. [PubMed: 10669571]

Silvestre JS, O'neill MF, Fernández AG, Palacios J. Effects of a range of dopamine receptor agonists and antagonists on ethanol intake in the rat. European Journal of Pharmacology. 1996; 318:257–265. [PubMed: 9016913]

Solinas M, Ferré S, Antoniou K, Quarta D, Justinova Z, Hockemeyer J, Pappas L, Segal P, Wertheim C, Müller C, Goldberg S. Involvement of adenosine A1 receptors in the discriminative-stimulus effects of caffeine in rats. Psychopharmacology. 2005; 179:576–586. [PubMed: 15696333]

Sprow GM, Thiele TE. The neurobiology of binge-like ethanol drinking: Evidence from rodent models. Physiology & Behavior. 2012; 106:325–331. [PubMed: 22245775]

Szumlinski KK, Diab ME, Friedman R, Henze LM, Lominac KD, Bowers MS. Accumbens neurochemical adaptations produced by binge-like alcohol consumption. Psychopharmacology. 2007; 190:415–431. [PubMed: 17225170]

Thorsell A, Johnson J, Heilig M. Effect of the Adenosine A2a Receptor Antagonist 3, 7-Dimethyl-Propargylxanthine on Anxiety-like and Depression-like Behavior and Alcohol Consumption in Wistar Rats. Alcoholism: Clinical and Experimental Research. 2007; 31:1302–1307.

Viner RM, Taylor B. Adult outcomes of binge drinking in adolescence: findings from a UK national birth cohort. Journal of epidemiology and community health. 2007; 61:902–907. [PubMed: 17873228]

Wickens J. Striatal dopamine in motor activation and reward-mediated learning: steps towards a unifying model. Journal of Neural Transmission/General Section JNT. 1990; 80:9–31.

Wilcox MV, Carlson VCC, Sherazee N, Sprow GM, Bock R, Thiele TE, Lovinger DM, Alvarez VA. Repeated Binge-Like Ethanol Drinking Alters Ethanol Drinking Patterns and Depresses Striatal GABAergic Transmission. Neuropsychopharmacology. 2014; 39:579–594. [PubMed: 23995582]

Williams AJ, Cronstein BN. The effect of A2A adenosine receptor activation on CC chemokine receptor 7 expression in human THP1 macrophages during inflammation. Inflammation. 2012; 35:614–622. [PubMed: 21739164]

Highlights

- The adenosine A_1 antagonist DPCPX significantly decreased binge-like alcohol intake
- The A_{2A} antagonist MSX-3 did not significantly influence alcohol or sucrose intake
- MSX-3 elicited particularly robust locomotor stimulation in mice consuming alcohol

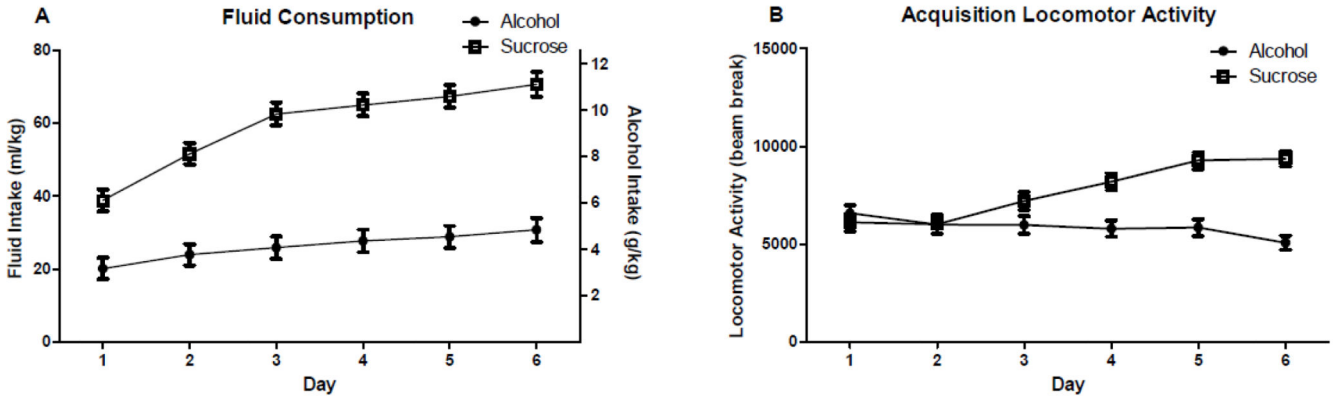

Figure 1.

A) Fluid intake for 20% (v/v) alcohol or 2% (w/v) sucrose and B) home cage locomotor activity during the 6-day DID acquisition period. *n*'s = 75-79. Subsequent dose group assignment for both drugs was counterbalanced on both of these measures so that no baseline differences existed.

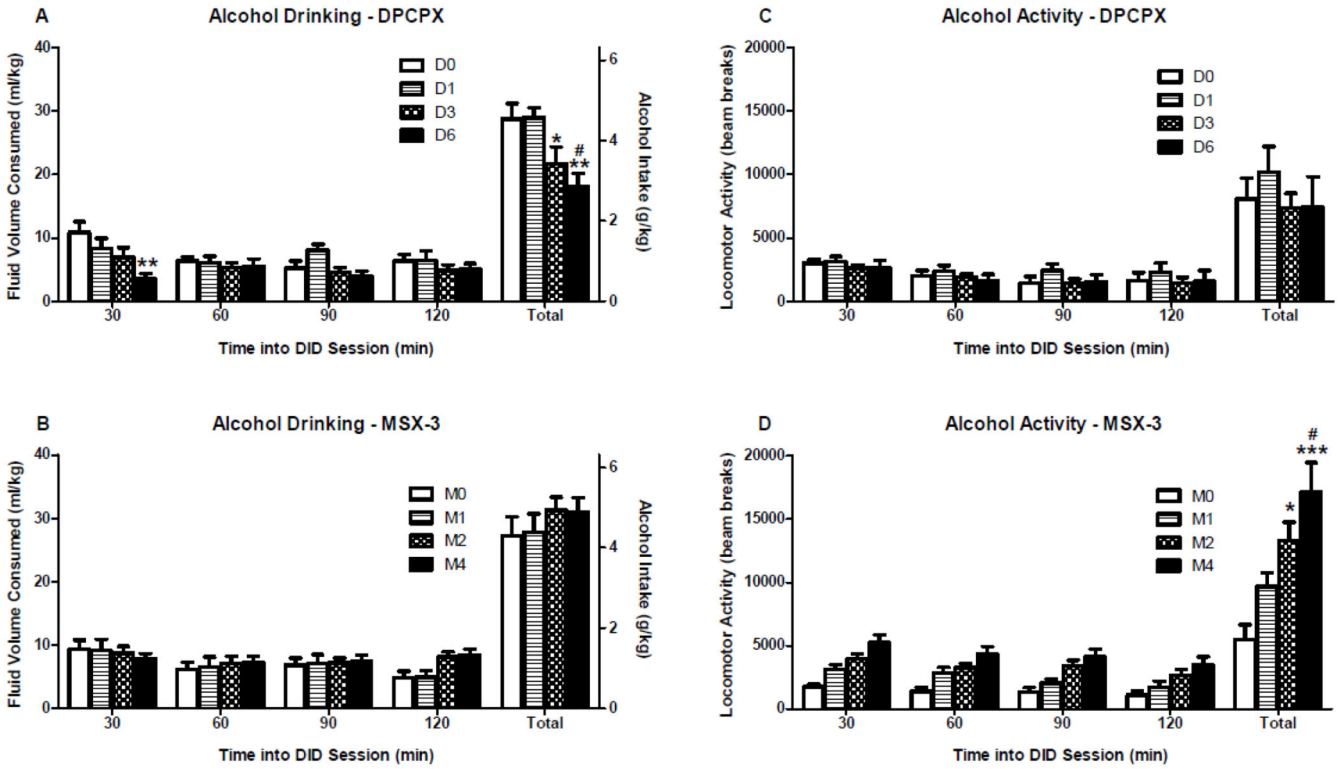

Figure 2.

Alcohol (20%) consumption following systemic administration of A) the A_1 antagonist DPCPX [0, 1, 3, 6 mg/kg; i.p.] or B) the A_{2A} antagonist MSX-3 [0, 1, 2, 4 mg/kg; i.p.] over the course of the 2-hr DID session on day 7. Home cage locomotor activity following administration of C) DPCPX and D) MSX-3 is also shown for the 2-hr DID session on day 7. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle control group; $^{\#}p < 0.05$ verus 1 mg/kg group. *n*'s = 8-12.

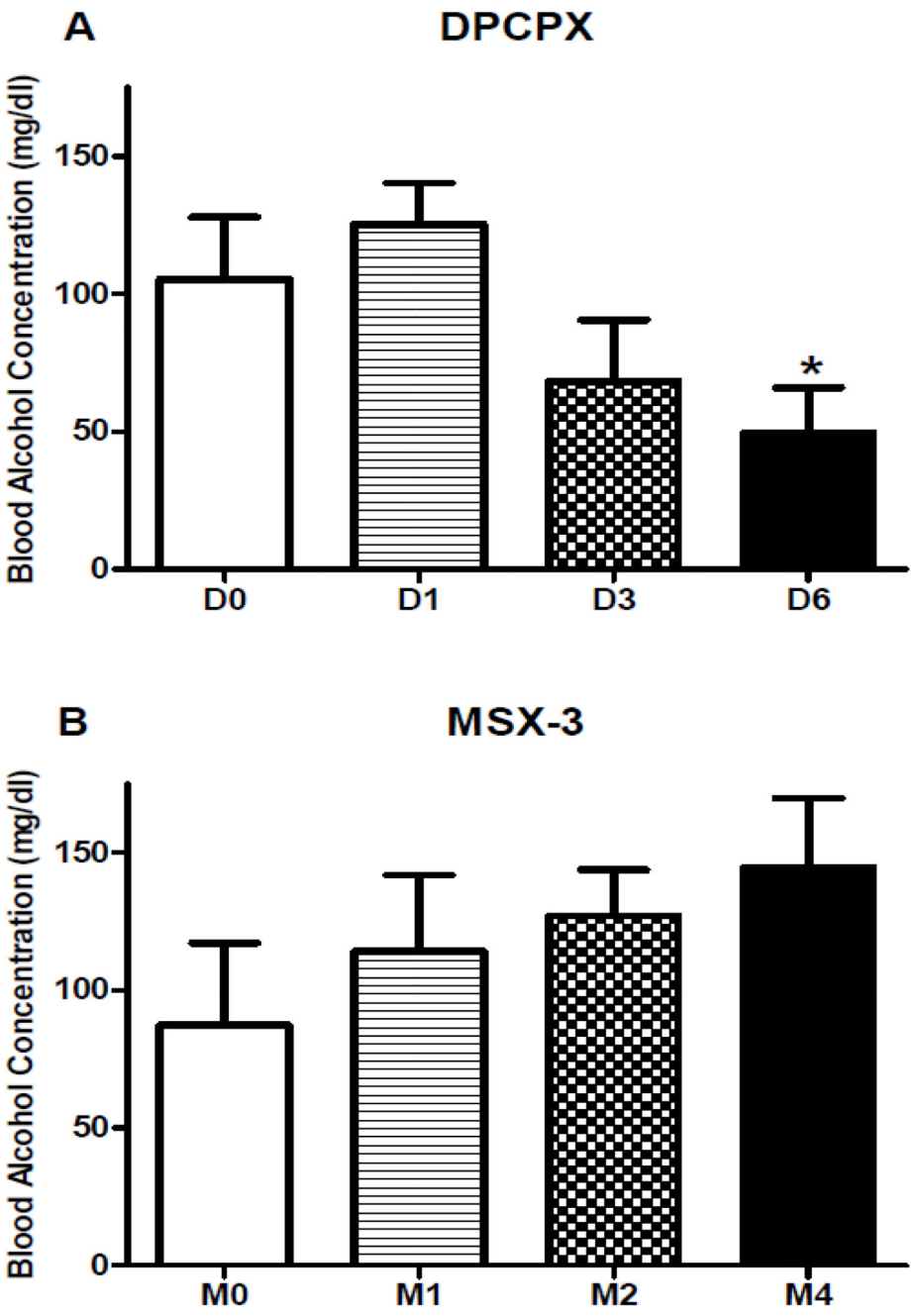

Figure 3.

Blood alcohol concentrations (BACs) following the 2-hr drug challenge DID session on day 7. BACs following A) DPCPX [0, 1, 3, 6 mg/kg; i.p.] or B) MSX-3 [0, 1, 2, 4 mg/kg; i.p.] administration. *$p < 0.05$ versus vehicle control group. n's = 8-12.

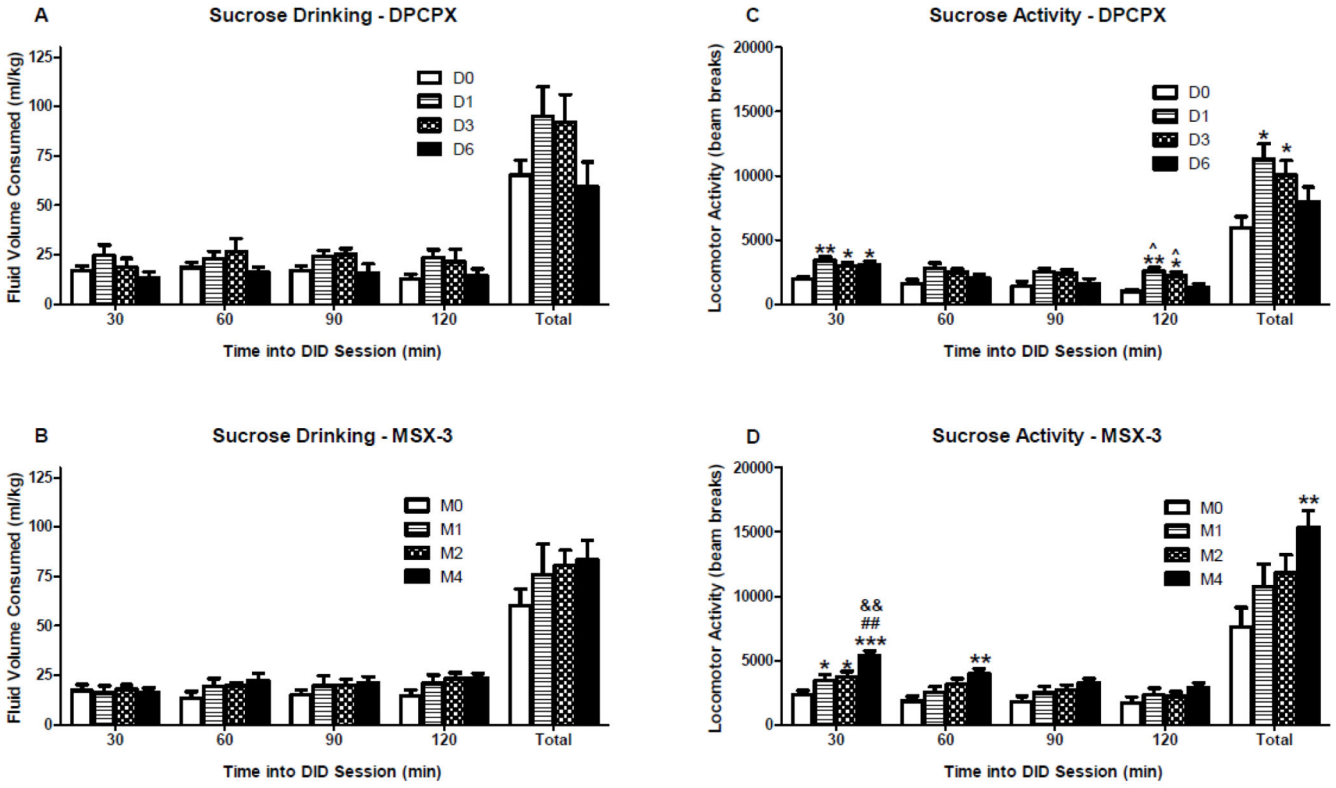

Figure 4.

Sucrose (2%) consumption following systemic administration of A) the A_1 antagonist DPCPX [0, 1, 3, 6 mg/kg; i.p.] or B) the A_{2A} antagonist MSX-3 [0, 1, 2, 4 mg/kg; i.p.] over the course of the 2-hr DID session on day 7. Home cage locomotor activity following administration of C) DPCPX and D) MSX-3 is also shown for the 2-hr DID session on day 7. *$p < 0.05$, **$p < 0.01$ versus vehicle control group. n's = 8-11.